Mater. Res. Soc. Symp. Proc. Vol. 845 © 2005 Materials Research Society AA5.47

Novel Angiogenic Compounds for Targeted Drug Delivery

Kristen A. Wieghaus[1], Scott M. Capitosti[2], Milton L. Brown[2], and Edward A. Botchwey[1]
[1]Department of Biomedical Engineering, [2]Department of Chemistry
University of Virginia
Box 800759, Health System
415 Lane Road, MR-5 Building
Charlottesville, VA 22908

ABSTRACT

Induction of angiogenesis is necessary for the success of engineered implantable tissues in order to meet oxygen and nutrient requirements of cells during tissue repair. Insufficient vascularization in bone graft reconstruction may impede healing and initiate hypoxic cell death at the interior of the implant. As a result, endogenous growth factors have been studied to enhance angiogenesis during wound repair. However, these peptide-based molecules are highly sensitive to processing that occurs during scaffold biomaterial fabrication and treatment for tissue engineering purposes. We report here the development of new small molecule regulators of angiogenesis that may circumvent the impediments associated with protein-based growth factor delivery. In this study, we report the design and evaluation of SC-3-143 as a regulator of endothelial function. We show that the compound significantly increases the formation of microvascular networks *in vitro*, and selectively enhances endothelial survivability by reducing endothelial cell death under serum deprived culture conditions.

INTRODUCTION

Formation of functioning microvascular networks is a complex process, with numerous growth factors that are required to initiate sprouting and remodeling of vessels[1,2,3] and to properly regulate cellular interactions. As a result, numerous therapeutic approcahes involving endogenous growth factor delivery have been proposed enhance angiogenesis during tissue repair. However, these biomolecules are highly sensitive to the thermal processing, sterilization, and exposure to solvents that occur during incorporation within scaffold biomaterials. These factors may limit the ability to obtain sufficient amounts of protein release necessary to obtain the desired biological responses when these growth factors are incorporated into and released from biomaterials.

In this study, we report the development of new small molecule stimulators of angiogenesis that are suited for targeted drug delivery from polymeric biomaterials. Specifically, we report on novel organic compounds, based on a thalidomide analogue that can be modified to exhibit pro-angiogenic activity. Thalidomide has clinical relevance as a sedative, but has more recently been shown to be antiangiogenic[4] and to function as an effective treatment for tumorigenic cancers.[5] We have developed SC-3-149 (results not shown) and SC-3-143 (see Figure 1), thalidomide analogues that *stimulate* endothelial cell proliferation.

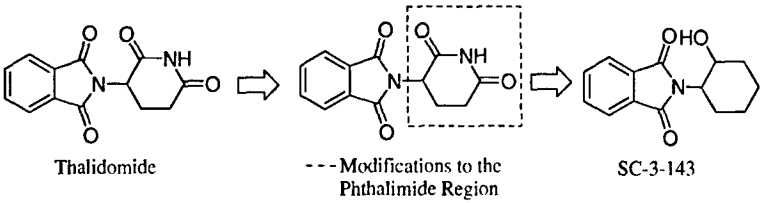


Figure 1. Design strategy for SC-3-143, a novel analogue of thalidomide.

We have previously shown that SC-3-149 enhances proliferation, survival, and capillary formation of human microvascular endothelial cells (HMVEC) *in vitro*.[6] In this study, we demonstrate that SC-3-143, a related compound, also augments microvascular cell survivability in serum-deprived conditions, and promotes rapid formation of vascular endothelial cords *in vitro*.

EXPERIMENTAL DETAILS

Cells were cultured on tissue-culture plates (Nunc) at 37° C in a humidified chamber with 5% CO_2. HMVEC (Cambrex) were cultured in EGM2-MV (bulletkit, BioWhittaker) supplemented as directed. Human osteoblast-like cells (SaOS-2) were cultured in M-199 media (Clonetics) with 10% FBS (Gibco), 5% penicillin/streptomycin, (Gibco), and 2.5% gentamicin (10mg/mL, MP Biomedical). Bovine aortic endothelial cells (BAEC) were cultured in DMEM supplemented with 10% FBS, 5 mM L-glutamine, 0.5% pen/strep, and 2.5% gentamicin.

For cell survival studies, HMVEC, BAEC, and SaOS-2 cells were seeded at sub-confluent densities and cultured on tissue-culture plastic. When confluency was reached, media was removed and the cells were rinsed. The samples were then replenished with culture media, serum-deprived media (SDM), SDM supplemented with 30 μM SC-3-143 and 0.6% DMSO delivery vehicle, SDM supplemented with 0.1% vascular endothelial growth factor (VEGF) solution, or SDM supplemented with 30 μM SC-3-143 and 0.1% VEGF solution. Serum-deprived media is composed as basal media with 0.5% FBS (Gibco). Wells that did not contain drug were treated with a 0.6% DMSO control. After 24 hrs of cultivation, cells were trypsinized and counted using a hemacytometer with trypan blue exclusion of dead cells.

To investigate the effects of SC-3-143 on capillary network formation, Matrigel (BD Biosciences) was crosslinked in a microwell plate at 37° C for 45 minutes. Subsequently, 10,000 HMVEC cells, supplemented with either 30 μM SC-3-143 or vehicle control, were plated on each sample. Samples were incubated 24 hours, and capillary network formation was inspected with an inverted light microscope at 10X and 40X magnification. The average number of tubular structures in each well was determined, defining a tubule as a multicellular structure with a length at least four times its width.[7]

RESULTS AND DISCUSSION

Our laboratories have combined tissue engineering and medicinal chemistry to investigate new therapeutic approaches in regenerative medicine. More specifically, we report here on a novel thalidomide analogue, SC-3-143, that has been developed for incorporation into and release from biomaterials. In this phase of our studies, we explore its angiogenic effect on endothelial function *in vitro*.

A small molecule compound capable of enhancing angiogenesis would have broad applications in tissue engineering. We envision the incorporation and local delivery of SC-3-143 and novel analogues into polymeric biomarterials such as the poly(α-hydroxy acids), where localized acidity profiles of polymers have been characterized.[8] These small molecules are well-suited for localized delivery from conventional scaffold biomaterials, and may provide more desirable release kinetics and local diffusion rates than a peptide-based growth factor. In addition, these molecules might better withstand scaffold fabrication processes that may inactivate peptide-based growth factors.[9,10]

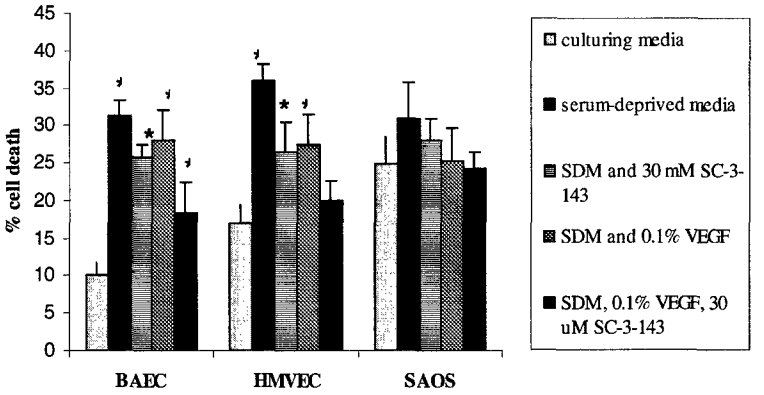
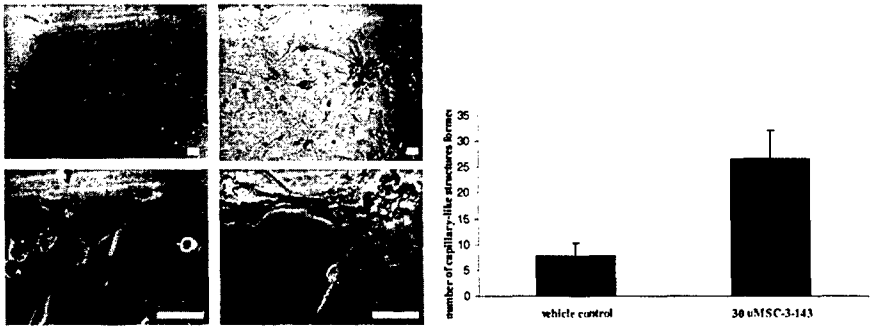


Figure 2. Results of the cell viability assay. "*" denotes statistically significant ($P<0.05$) cell necrosis, as compared with cells grown in culturing media.

In this study, we show that the addition of 30 μM SC-3-143, delivered concurrently with 0.1% VEGF, to serum-deprived media significantly increases the cell viability of HMVEC and BAEC cells after only 24 hours, as shown in Figure 2. In future studies, we will examine the effects of SC-3-143 and novel analogues to the reduce cytoxicity associated caused by release local acidity. In additon, a compound like SC-3-143 might also stimulate formation of vascular networks within implanted scaffold biomaterials. Figure 3A shows results obtained after cultivating HMVEC cells in Matrigel for 24 hours with and without supplementation with SC-3-143. These studies demonstrate that cells supplemented with SC-3-143 (panels 2 and 4) exhibit more cell-cell associations and tube-like structures than control samples (panels 1 and 3). Moreover, quantitive analysis shows



A B

Figure 3. A) Light micrographs of cells cultured in Matrigel with DMSO vehicle control (panels 1 and 3) or 30 μM SC-3-143 (panels 2 and 4) after 24 hours (scale bar = 20 μm). B) Capillary network formation in each HMVEC sample after 24 hours, with either 30 μM SC-3-143 or vehicle control. "*" denotes statistically significant ($P<0.05$) capillary formation as compared with the control.

that SC-3-143 induces significantly more capillary network formation than vehicle controls (Figure 3B).

CONCLUSION

We believe that the development of SC-3-143 and other novel analogues will lead to new treatment modalities for therapeutic angiogenesis and tissue engineering. In this first phase of our studies, we demonstrate that SC-3-143 increases endothelial cell viability under nutrient deprived conditions and induces capillary network formation in three-dimensional cultures. Our goal is to release SC-3-143 and novel analogue compounds from within 3-D scaffolds to promote graft vascularization. Ultimately, such small molecule inducers of angiogenesis may possess several potential advantages over use endogenous growth factors commonly proposed for this purpose, such as reduced cost, precise measurements of delivered drug, and ability to withstand polymer processing. Moreover, we belive that these studies will elucidate new approaches to promote angiogenesis and improve bony repair potential of tissue-engineered bone grafts.

[1] Carmliet, P. (2000) *Nat. Med.* **6**, 1102-1103.
[2] Ferrara, N., & Alitalo, K. (1999) *Nat. Med.* 5, 1359-1364.
[3] Yancopoulos, G.D., Davis, S., Gale, N.W., Rudge, J.S., Wiegand, S.J., & Holash, J. (2000) *Nature* **407**, 242-248.
[4] D'Amato, R.J., Loughnan, M.S., Flynn, E., & Folkman, J. (1994) *Proc. Natl. Acad. Sci. USA* **91**, 9, 4082-4085.
[5] Rajkumar, S.V., Dispenzieri, A., Fonseca, R., Lacy, M.Q., Geyer, S., Lust, J.A., Kyle, R.A., Griepp, P.R., Gertz, M.A., & Witzig, T.E. (2001) *Leukemia* **15**, 8, 1274-1276.
[6] Wieghaus, K.A., Capitosti, S.M., Blackman, B.R., Brown, M.L., and Botchwey, E.A. Submitted work.
[7] Chen, J., Wan, X., Zhu, J., Shang, Y., Guo, X., & Sun, J. (2004) *J. Cardiovasc. Pharmacol.* **43**, 3, 347-352.
[8] Sung, H.-J., Meredith, C., Johnson, C., & Galis, Z.S. (2004) *Biomaterials* **25**, 5735-5742.
[9] Cleland, J.L., Powell, M.F., & Shire, S.J. (1993) *Crit. Rev. Ther. Drug. Carrier Sys.* **10**, 307-377.
[10] Peters, M.C., Polverini, P.J., & Mooney, D.J. (2002) *J. Biomed. Mat. Res.* **60**, 668-678.